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Bacteroides gingivalis-specific serum IgG and IgA subclass antibodies in periodontal diseases

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SUMMARY

The level of serum IgM, IgG and IgA antibodies including IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2 subclass-specific antibodies to *Bacteroides* (*Porphyromonas*) *gingivalis* fimbriae and to lipopolysaccharide (LPS) were analysed in patients with different forms of periodontal disease (PD) and control subjects by ELISA. Among PD subjects, sera obtained from adult periodontitis (AP), rapidly progressive periodontitis (RPP) and gingivitis contained high titres of fimbriae-specific IgG antibodies (7500–15000 ELISA units) followed by IgA (90–700 units) and IgM (30–90 units). In contrast, sera from localized juvenile periodontitis (LJP) subjects exhibited much lower titres of fimbriae-specific IgG (89 ± 11 units), IgA (31 ± 5 units) and IgM (17 ± 3 units) antibodies. A similar response pattern was also seen in sera from normal subjects aged 35–41 years who practice normal oral hygiene, while sera of younger adults (aged 18–24) with superior hygiene did not have any antigen-specific antibodies. Analysis of IgG subclass anti-fimbriae responses revealed that the major response was IgG3 followed by IgG1, IgG2 and IgG4 in AP, RPP and gingivitis. Although lower, a similar pattern of IgG subclass titre was seen in LJP and normal subjects aged 35–41 years. When IgA subclass responses were measured in AP and RPP, higher titres of the fimbriae-specific response were noted with IgA1 when compared with IgA2. However, lower but approximately equal levels of fimbriae-specific IgA1 and IgA2 titres were seen in other PD groups. When anti-*B. gingivalis* LPS-specific responses were measured, the sera of AP patients contained high levels of IgG antibodies (2265 ± 224 units) followed by IgA (411 ± 90 units) and IgM (214 ± 56 units). Further, IgG anti-LPS responses were mainly IgG2 followed by IgG4, IgG3 and IgG1. For IgA subclass responses, higher titres of anti-LPS-specific antibodies were noted in IgA2 subclass over IgA1. These results showed that higher anti-*B. gingivalis* antibody responses occur in PD when compared with healthy individuals and protein and lipid-carbohydrate antigens of *B. gingivalis* induce distinct patterns of antigen-specific IgG and IgA subclass responses.

Keywords *Bacteroides gingivalis* (*Porphyromonas gingivalis*) fimbriae lipopolysaccharide IgA subclasses IgG subclasses periodontal diseases

INTRODUCTION

The separate subclasses of human IgG and IgA (IgG1, IgG2, IgG3, IgG4, and IgA1 and IgA2) are determined by unique primary amino acid differences in the constant regions of the heavy chain. Each subclass has a characteristic biological and functional property (reviewed by Burton, Gregory & Jefferis, 1986; Mestecky & Russell, 1986). It is now generally accepted that IgG1 and IgG3 are much more effective than the other two IgG subclasses in triggering effector functions. In this regard, the Fc of IgG1 and IgG3 has a high affinity for complement (C) proteins, specifically C1q, and when complexed with antigen activates the classical C pathway (Burton *et al.*, 1986). Although IgG3 was most efficient for C1q binding among the IgG subclasses, IgG1 antibodies were more effective in lysis of target cells (Bindon *et al.*, 1988). On the other hand, IgG2 antigen complexes are less efficient in binding C1 proteins and IgG4 does not bind and initiate the classical C pathway (Burton *et al.*, 1986). IgG1 and IgG3 also exhibit a strong binding affinity for the FcγRI on monocytes and macrophages, and for the FcγRIII on neutrophils, natural killer (NK) cells and tissue macrophages, and thus are important in opsonization and phagocytosis (Unkeless, Scigliano & Freedman, 1988). Unlike IgG subclasses, the effector functions associated with IgA1 and IgA2

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are less well characterized; however, it has been shown that IgA mediates protection against microorganisms by a variety of mechanisms. These include direct killing, agglutination, prevention of attachment and invasion, inactivation of bacterial enzymes, opsonization and cell-mediated killing (reviewed by Childers, Bruce & McGhee, 1989). In contrast to IgG, IgA is more resistant to cleavage by proteolytic enzymes (Mestecky & Killian, 1985). Specific bacterial proteases produced by several pathogenic bacteria selectively cleave IgA1 into Fab and Fc fragments; however, IgA2 is resistant to these proteases (Mestecky & Killian, 1985). These differences in susceptibility of IgA subclasses to bacterial IgA1 proteases could profoundly influence the biologic effector functions of IgA, especially during the course of an infection.

Periodontal diseases (PD) can generally be classified into several forms, including adult periodontitis (AP), rapidly progressive periodontitis (RPP), and localized juvenile periodontitis (LJP), and gingivitis (reviewed by Page & Schroeder, 1982). It is now generally accepted that the destructive manifestations of local cellular responses to specific oral bacteria at the local site induce the inflammation associated with PD. In this regard, *Bacteroides* (*Porphyromonas*) *gingivalis* has been consistently isolated from the localized region of AP and in some instances from LJP, while *Actinobacillus actinomycetemcomitans* is usually associated with LJP (Slots & Listgarten, 1988). Strong evidence for an association between a specific bacterium with ensuing PD has been provided by analysis of serum antibodies reactive with the oral bacterium. It was shown that patients with AP exhibit higher serum antibody levels to *B. gingivalis* than do control subjects (Ebersole *et al.*, 1982b, 1986; Farida *et al.*, 1986; Naito, Okuda & Takazoe, 1984, 1987; Vincent *et al.*, 1985). Further, elevated levels of serum antibodies to *A. actinomycetemcomitans* was also evident in LJP patients (Ranney *et al.*, 1982; Ebersole *et al.*, 1982a; Genco, Zambon & Murray, 1985; Farida *et al.*, 1986). In these studies, the major antigen-specific antibody was of the IgG isotype followed by IgA or IgM. High titres of IgG antibodies have been considered to be important in the destructive events which occur in the disease site, since IgG antibodies exhibit effector functions that affect the induction of inflammatory responses directly.

The subclasses of antibodies to PD-associated bacteria have not been studied, and it is difficult to assess the specific effector functions which these antibodies mediate. In order to understand this better, we analysed the isotype and subclass of antibodies reactive with *B. gingivalis* fimbriae and lipopolysaccaride (LPS) in sera of patients with various forms of PD when compared with healthy controls. Our results provide evidence that sera obtained from various forms of PD (except LJP) display high levels and distinct patterns of fimbriae-specific IgG subclass antibodies and lower but significant IgA. Further, lower levels of LPS-specific IgG and IgA antibodies were seen with patterns totally distinct from fimbriae responses.

MATERIALS AND METHODS

Cultivation of B. gingivalis

B. gingivalis 381 was grown in GAM broth (Nissui, Tokyo, Japan) supplemented with haemin (5 mg/*l*; Wako Pure Chemical Industries, Osaka, Japan) and menadione (10 μg/*l*; Wako) as described previously (Ogawa *et al.*, 1989a). After 26 h of incubation at 37°C in an anaerobic chamber (Model 1024, Forma Scientific, Marietta, OH) containing 5% CO_2, 5% H_2 and 90% N_2, the bacteria were harvested by centrifugation at 10000 ***g*** for 30 min at 25°C.

Preparation of B. gingivalis *fimbriae*

Large-scale production of fimbriae was made by modifications of a method previously described (Ogawa *et al.*, 1989a). Bacteria were harvested from a 16-*l* culture (120 g, wet weight) and suspended in 2 *l* of 20 mM Tris–HCl (pH 7·4), 0·15 M NaCl, and 10 mM $MgCl_2$, and aliquots (200 ml each) of the suspension were gently pipetted. The aliquots were then combined, gently mixed with a stirring bar for 15 min at 25°C, and centrifuged at 10000 ***g*** for 30 min at 25°C . The supernatant fluid was collected and ammonium sulphate was added to a 40% saturation. The precipitate was obtained by centrifugation, resuspended in 50 ml of 20 mM Tris-HCl buffer (pH 8·0), and dialysed against 25 *l* of the same buffer. The dialysate was clarified by centrifugation (10000 ***g*** for 15 min), and the supernatant was applied to a column (5 × 15 cm) of DEAE–Sepharose Fast Flow (Pharmacia, Uppsala, Sweden) which had been equilibrated with the same buffer. The column was washed with 1·5 *l* of 20 mM Tris–HCl buffer (pH 8·0) and eluted with a stepwise gradient of 0–0·15 M NaCl in 20 mM Tris-HCl buffer (pH 8·0). The fimbriae protein was eluted from the column at a concentration of 0·15 M NaCl of the elution buffer. The fractions containing the fimbriae protein were combined and concentrated by ammonium sulphate precipitation and dialysed against 10 *l* of 20 mM Tris–HCl buffer (pH 8·0). A total of 130 mg of the purified protein was obtained from 16 *l* of cultured *B. gingivalis* 381. SDS–PAGE with 1·0-mm-thick slab gel (12%) revealed a distinct and single band at 41 kD. The purity of the fimbriae protein was also determined by double diffusion in an agar plate with rabbit anti-fimbriae and anti-*B. gingivalis* whole cell antibodies as well as with monoclonal anti-fimbriae antibodies. No other contaminating proteins were detectable.

B. gingivalis *lipopolysaccharide preparation*

LPS was prepared from *B. gingivalis* by the standard hot phenol water method as previously described (Koga *et al.*, 1984). Briefly, lyophilized *B. gingivalis* cells were suspended in 350 ml of pyrogen-free water and 350 ml of 90% phenol. The mixture was stirred vigorously at 65°C for 5 min and then centrifuged at 5000 ***g*** for 30 min. The aqueous phase was removed, and the phenol phase and insoluble precipitates were combined, dialysed extensively against distilled water, lyophilized, and stored at −20°C until used. The biochemical and biological properties of phenol-water-extracted LPS from *B. gingivalis* are described elsewhere (Koga *et al.*, 1984).

Analysis of anti-fimbriae and anti-LPS antibodies

The isotype and level of antibodies specific for *B. gingivalis* fimbriae and LPS in serum specimens were determined by a modified ELISA (Voller, Bidwell & Bartlett, 1980). Briefly, a 100-μl solution of fimbriae antigen (10 μg equivalent of BSA/ml) suspended in 0·1 M carbonate buffer (pH 9·6) was added to wells of a flat-bottomed micro-ELISA plate (M129A; Dynatech Laboratories, Sussex, UK). In some experiments, *B. gingivalis* LPS preparations were coupled with poly-L-lysine as described (Gray, 1979), and plates were coated with 100 μg/ml of LPS. The plates were then incubated at 4°C for 16 h, and were repeatedly washed with PBS containing 0·05% Tween 20 and 0·02%

sodium azide (PBS-T) to remove the unbound fimbrial antigen. Following the final wash, 100 μl of serum specimens appropriately diluted (1/1 to 1/100000) with PBS-T were added to each well. Following incubation at 4°C for 16 h, the plates were washed three times with PBS-T. Alkaline phosphatase-labelled goat anti-human mu, gamma or alpha antibodies (diluted 1/1000) (Zymed Laboratories, San Francisco, CA) were then added to the appropriate wells. Following 2 h incubation at 37°C, plates were washed and *P*-nitrophenylphosphate (104 phosphatase substrate, Sigma Chemical Company, St Louis, MO) dissolved in diethanolamine solution (pH 9·8) was added to individual wells at a concentration of 100 μg/well. The colour reaction was stopped by addition of 50 μl of 3 N sodium hydroxide after 30 min incubation at room temperature. The extent of colour was determined by a Titertek Multiskan MC photometer (Flow Laboratories, McLean, VA) at 405 nm.

Antibody levels in the sera from patients and normal subjects were expressed as ELISA units (EU) which were defined by use of a reference curve obtained from linear regression analysis. Nine sera that contained *B. gingivalis* fimbriae- and LPS-specific antibodies were selected from normal subjects aged 35–41 years. These samples were pooled and used as a reference serum. Antigen-specific standard titration curves were obtained for IgM, IgG and IgA isotypes as well as IgG and IgA subclasses by plotting the mean absorbance of the reference serum in serial dilutions of 1/2–1/16384. Individual sigmoid dose–response curves were then converted to linear equation of the regression ($Y = A + BX$) by log–logit transformation using the formula (Rodbard, Bridson & Rayford, 1969):

$$X = \log_{10} \text{Dilution},$$
$$Y = \text{logit}\ (OD_{405}) = \log_{10}\left(\frac{OD_{405} - U}{M - OD_{405}}\right)$$

Individual serum samples from different patient groups were diluted from neat to 1/100000 for the determination of the isotype and subclass of antigen-specific antibody titres. Following OD_{405} determination of these samples by use of a titertek Multiskan MC photometer (Flow), the EU of individual samples were then calculated by relating the optical density values from individual serum specimens to the reference curve using the formula of EU = (dilution of samples/dilution factor obtained from the reference curve) × 100.

IgG and IgA subclass-specific antibodies

To determine the titre of antigen-specific IgG subclass antibodies, we used the monoclonal mouse anti-human gamma subclass specific antibodies, HP6012 (γ1), HP6014 (γ2), HP6047 (γ3) and HP6022 (γ4) as previously described (Ogawa *et al.*, 1989b). These monoclonal antibodies were tested for specificity with human myeloma IgG1, IgG2, IgG3 and IgG4 proteins (Ogawa *et al.*, 1989b). Serum samples from patients or normal subjects were applied on antigen pre-coated wells as described above and after incubation at 4°C for 16 h, the appropriate monoclonal antibodies were added to wells (1 μg/100 μl per well) and plates were further incubated for 2 h at 37°C. Alkaline phosphatase-labelled goat IgG anti-mouse immunoglobulin (Zymed) was added (1/1000 diluted) and plates were incubated for 2 h at 37°C. Colour was developed as described above. A similar assay was performed to determine the titre of antigen-specific IgA1 and IgA2 antibodies. Briefly, purified mouse monoclonal anti-human IgA1 or IgA2 antibodies (1 μg/100 μl per well) were used as the detection antibody. These antibodies were kindly provided by Dr J. Radl, Institute of Experimental Gerontology, Rijswijk, The Netherlands and Dr J. Mestecky, University of Alabama at Birmingham, and their characterization was described elsewhere (Crago *et al.*, 1984; Ogawa *et al.*, 1989a, 1989b). IgG and IgA subclasses of antigen-specific EU were also defined by use of a reference curve obtained from linear regression analysis as described above.

Serum samples from PD patients and normal subjects

Serum samples were collected from PD patients and normal subjects at the University of Alabama at Birmingham, the private dental clinic headed by Dr James E. Roberts in Birmingham and at the Dental School of Osaka University in Japan. The study was discussed with each patient and healthy subjects and they all agreed to allow their blood samples to be used in this study. Blood (3–5 ml) was drawn by venepuncture and serum samples were obtained by centrifugation following clotting. In some cases, plasma was obtained since the peripheral blood was subjected to a Ficoll–Hypaque gradient to isolate mononuclear cells for other studies. All serum and plasma samples were stored at −70°C until used.

A total of six groups consisting of four different types of PD and two normal populations were used in this study. Clinical criteria for various types of PD used in this study has been described by others (Page & Schroeder, 1982). The AP group consisted of 23 patients (aged 35–42 years) and exhibited alveolar bone loss with multiple vertical osseous defects in the molar area and in the anterior segment. Eighteen patients younger than 35 years (aged 24–33 years) who exhibited severe generalized bone loss without any consistent pattern of distribution were grouped as RPP. A LJP group consisted of five patients (aged 14–21 years) exhibiting vertical alveolar bone resorption in incisors and/or first molars. Fifteen patients (19–30 years old) exhibiting gingival inflammation around several teeth but showing no evidence of radiographic alveolar bone loss, no probing attachment loss, and no periodontal pockets deeper than 3 mm were used as the gingivitis group. Normal adults consisted of two groups: 12 subjects with healthy gingival tissues and with excellent oral hygiene (aged 18–24), and a second group of 21 subjects (35–41 years old) with occasional slight–mild gingival inflammation.

RESULTS

Analysis of B. gingivalis *fimbriae-specific antibodies*

The isotype and level of *B. gingivalis* fimbriae-specific antibodies were assessed in sera of patients with four different types of PD by ELISA. As controls, sera from two groups of normal subjects were employed. Among the PD groups, high titres of fimbriae-specific antibodies were observed in sera obtained from patients with AP, RPP and gingivitis (Fig. 1a), while serum samples of LJP subjects contained lower levels of fimbriae-specific antibodies. The major isotype of *B. gingivalis* fimbriae-specific antibodies was IgG followed by IgA and IgM. Further, pre-incubation of sera from patients with AP, RPP and gingivitis with *B. gingivalis* removed fimbriae-specific IgM, IgG and IgA antibodies (data not shown). When sera from the two normal groups were examined, the older group (aged 35–41 years) contained some fimbriae-specific antibodies and the titre was similar to that of subjects with LJP. On the other hand, no

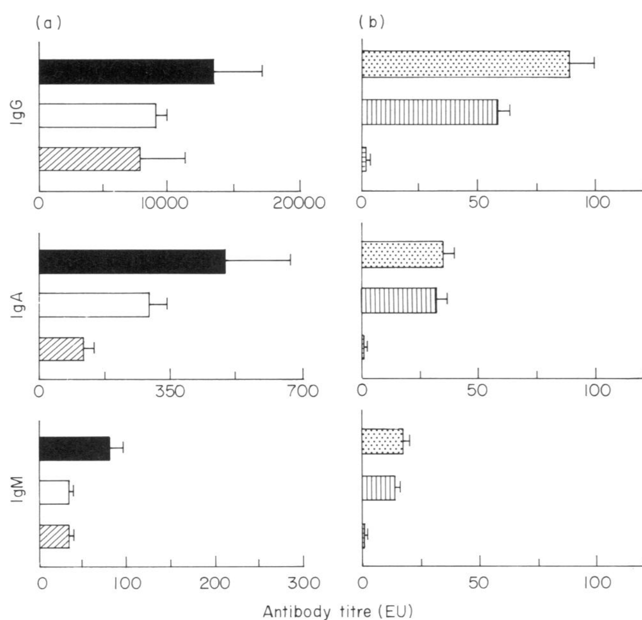

Fig. 1. *B. gingivalis* fimbriae-specific IgG, IgA and IgM antibodies in sera from subjects with: (a) adult periodontitis (■), rapidly progressive periodontitis (□) and gingivitis (▨); and (b) local juvenile periodontitis (▩), normal subjects aged 35–41 years (■) and normal subjects aged 18–24 years (■). Values are in ELISA units (EU) relative to the reference serum + s.e.m.

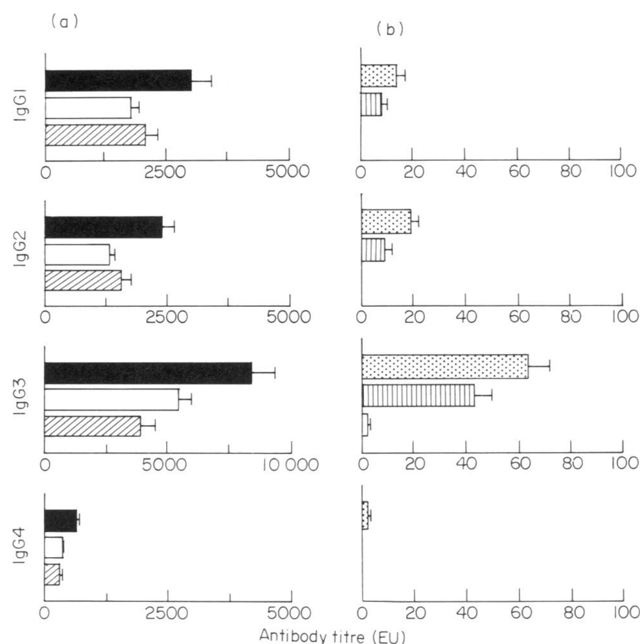

Fig. 2. Comparison of *B. gingivalis* fimbriae-specific IgG subclass antibodies in sera from subjects with: (a) adult periodontitis (■), rapidly progressive periodontitis (□) and gingivitis (■); and (b) local juvenile periodontitis (▩), normal subjects aged 35–41 years (■) and normal subjects aged 18–24 years (■). Values are in ELISA units (EU) relative to the reference serum + s.e.m.

detectable fimbriae-specific antibodies were found in sera of the younger normal subjects (aged 18–24) (Fig. 1b). These results show that sera isolated from AP, RPP and gingivitis contain high titres of fimbriae-specific IgG antibody with significant levels of IgA. On the other hand, sera of LJP which is often associated with *A. actinomycetemcomitans* infection, had much lower levels of *B. gingivalis* fimbriae-specific antibodies which were comparable to those seen in sera of the older healthy controls. The young adult subjects with excellent oral hygiene did not have any fimbriae-specific antibodies.

IgG subclass distribution of fimbriae-specific antibodies

Since fimbriae-specific antibodies were predominantly of IgG isotype in patients with PD, the titre of IgG subclass distribution was examined in sera of patients with AP, RPP and gingivitis. Increased levels of IgG1, IgG2, IgG3 and IgG4 fimbriae-specific antibody titres were seen in AP, RPP and gingivitis when compared with LJP and normal control groups (aged 18–24 and 35–41 years) (Fig. 2). In general, a 200–300-fold higher titre of fimbriae-specific antibodies was observed in the individual IgG subclasses. A distinct pattern of antigen-specific IgG subclass antibody titre was found in sera of AP, RPP and gingivitis where the IgG3 subclass was highest followed by IgG1, IgG2 and IgG4 (Fig. 2a). A similar pattern of IgG subclass titre was also seen in serum samples of LJP and normal subjects aged 35–41, although the titres of fimbriae-specific antibodies were much lower than those of AP, RPP and gingivitis (Fig. 2b). It was also important to note that the sum of the fimbriae-specific IgG1–IgG4 titres in each sample was approximately equal to the total antigen-specific IgG antibody titre (Figs 1 and 2). These results show that sera of patients with AP, RPP and gingivitis exhibit higher levels of fimbriae-specific IgG subclass antibody titres with a pattern of IgG3»IgG1 > IgG2 > IgG4.

Fimbriae-specific antibodies in IgA1 and IgA2 subclasses

Since higher fimbriae-specific IgA antibody responses were also observed in patients with AP, RPP and gingivitis when compared with healthy subjects (Fig. 1), IgA fimbriae-specific antibody titres in sera of these patients were tested for their IgA subclass distribution. Higher titres of fimbriae-specific IgA antibodies were seen in the IgA1 subclass when compared with IgA2 in the AP and RPP groups (Fig. 3). On the other hand, equal titres of IgA1 and IgA2 antibodies were noted in sera of patients with gingivitis. Although titres of fimbriae-specific IgA antibodies were low in sera of LJP and normal subjects aged 35–41, it was interesting that titres of antigen-specific IgA1 and IgA2 antibodies were similar. As with the IgG subclasses, the sum of the antigen-specific IgA1 and IgA2 titres were approximately equal to the fimbriae-specific IgA antibody titre (Figs 1 and 3). These results suggest that two different patterns of fimbriae-specific IgA1 and IgA2 responses occur in PD and normal subjects, where higher IgA1 responses are seen in the advanced stage of PD (e.g. AP and RPP) while equal levels of IgA subclasses are found in the early stage of PD (e.g. gingivitis), *B. gingivalis* non-associated PD (e.g. LJP) and healthy subjects aged 35–41.

Analysis of B. gingivalis *LPS-specific antibodies*

Since patients with AP always possess high titres of fimbriae-specific antibodies, it was interesting to determine the isotype and level of LPS-specific antibodies in serum samples. Although

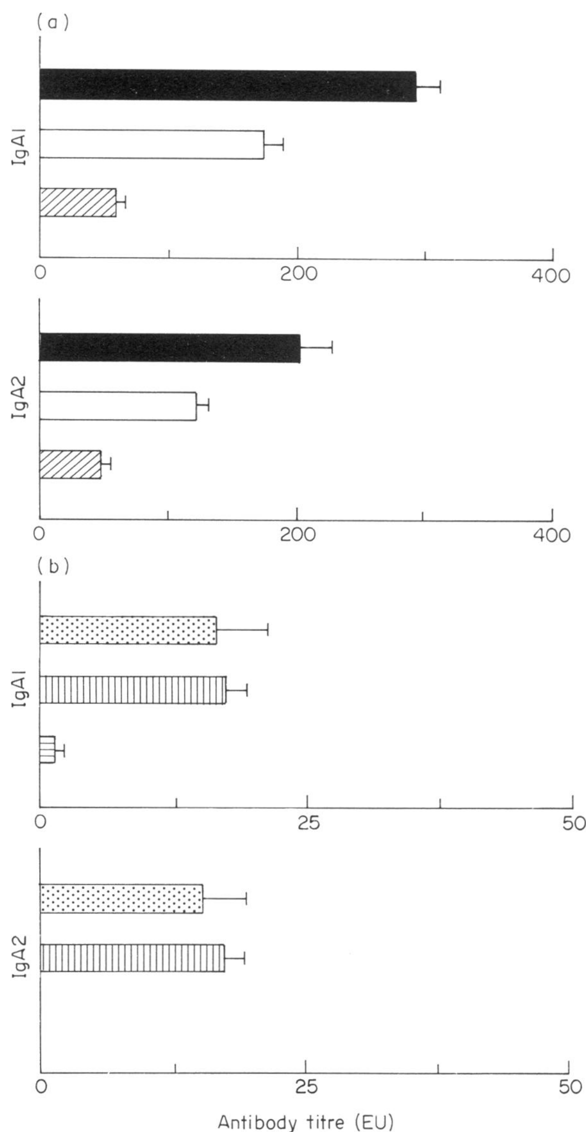

Fig. 3. Distribution of *B. gingivalis* fimbriae-specific IgA1 and IgA2 antibodies in sera from subjects with: (a) adult periodontitis (■), rapidly progressive periodontitis (□) and gingivitis (■); and (b) local juvenile periodontitis (▩), normal subjects aged 35–41 years (■) and normal subjects aged 18–24 years (■). Values are in ELISA units (EU) relative to the reference serum + s.e.m.

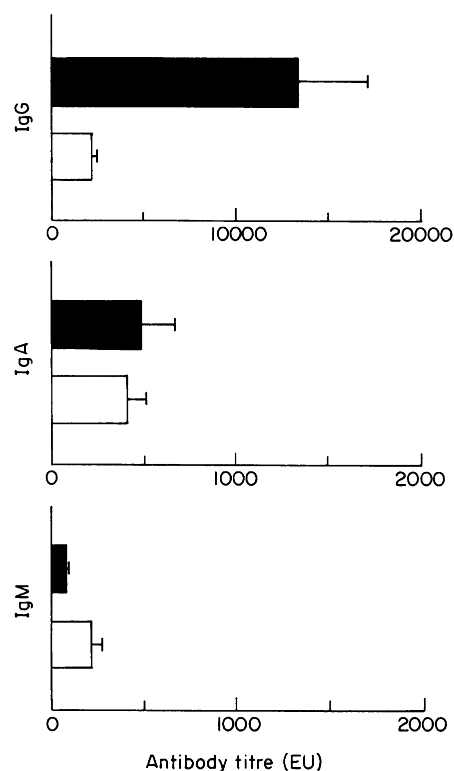

Fig. 4. *B. gingivalis* LPS-specific IgG, IgA and IgM antibodies in sera of patients with adult periodontitis. The level of LPS-specific antibodies were determined by ELISA. For comparison, the titre of fimbriae-specific IgM, IgG and IgA antibody from the same patients were also listed. The values are expressed as ELISA units (EU) relative to a serum reference ± s.e.m. □, LPS; ■, fimbriae.

titres of IgG LPS-specific antibody were lower when compared with fimbriae-specific responses, a similar pattern of isotype response was noted (IgG, 2265 ± 224 U, followed by IgA, 411 ± 90 U, and IgM, 214 ± 56 U) (Fig. 4). Further, LPS-specific IgG, IgA and IgG antibodies were removed by preincubation of AP serum with *B. gingivalis* (data not shown). Analysis of IgG subclasses revealed that the titre of IgG2 anti-LPS responses were highest, followed by IgG4, IgG3 and IgG1 (Fig. 5). When the titre of LPS-specific IgA subclass distribution was examined in sera of AP patients, higher titres were noted in IgA2 when compared with IgA1 (Fig. 6). Thus, anti-LPS responses were mainly of the IgG2 and IgA2 subclasses.

DISCUSSION

A potential role for *B. gingivalis*-specific antibodies in the development of PD was suggested by earlier studies where both serum and gingival crevicular fluid antibody levels often correlated with disease activity in patients with AP (reviewed by Slots & Listgarten, 1988). Moreover, increased levels of IgG antibodies to whole cells or to protein and carbohydrate antigens of *B. gingivalis* were detected in both serum and gingival crevicular fluid of patients with AP (Ebersole *et al.*,1982b, 1986; Vincent *et al.*, 1985; Farida *et al.*, 1986; Naito *et al.*, 1984, 1987). However, no studies have examined IgG and IgA subclass responses to purified antigens of PD-associated bacteria. Therefore the present study was undertaken to analyse the distribution and levels of IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2 antibody responses to *B. gingivalis* fimbriae and LPS in sera obtained from patients with four different types of PD—AP, RPP, gingivitis, and localized LJP, and compared with two groups of healthy subjects by using human IgG and IgA subclass specific monoclonal antibodies and ELISA.

Isotype analysis of *B. gingivalis* fimbriae-specific antibodies revealed that the major response was IgG followed by IgA and IgM (Fig. 1), a finding in agreement with earlier studies (Ebersole *et al.*, 1982b, 1986; Naito *et al.*, 1984, 1987; Vincent *et al.*, 1986). It is interesting that sera from patients with RPP and gingivitis also have high titres of IgG and lower but significant

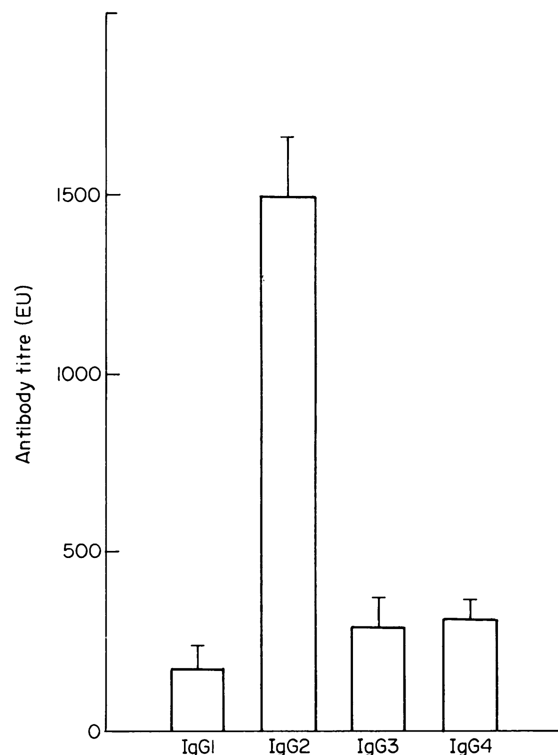

Fig. 5. Analysis of IgG subclass distribution in LPS-specific responses in sera of patients with adult periodontitis. The level of LPS-specific IgG1, IgG2, IgG3 and IgG4 were determined by ELISA. The values are expressed as ELISA units (EU) relative to a serum reference ± s.e.m.

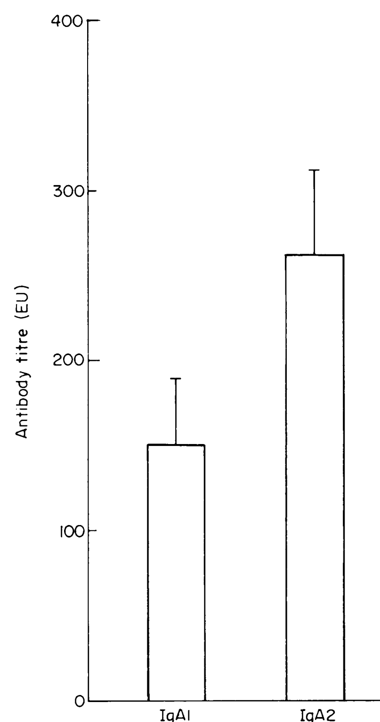

Fig. 6. Distribution of *B. gingivalis* LPS-specific IgA1 and IgA2 antibodies in sera of patients with adult periodontitis. The level of LPS-specific IgA1 and IgA2 were determined by ELISA. The values are expressed as ELISA units (EU) relative to a serum reference ± s.e.m.

levels of IgA fimbriae-specific antibodies (Fig. 1). On the other hand, serum samples obtained from LJP exhibited remarkably lower levels of *B. gingivalis* fimbriae-specific antibodies. The levels of fimbriae-specific antibodies in LJP patients were comparable to sera of normal subjects aged 35–41 years who practiced normal oral hygiene and exhibited occasional slight to mild gingivitis. However, the young adult population (aged 18–24 years) with excellent oral health did not have any detectable fimbriae-specific antibodies (Fig. 1). These observations clearly support the concept that *B. gingivalis* is associated with PD, including the AP, RPP and gingivitis forms. However, sera of patients with other types of PD such as LJP, which are mainly associated with *A. actinomycetemcomitans*, have low levels of *B. gingivalis* fimbriae-specific serum antibodies. These findings were supported by work which showed that significantly increased *B. gingivalis*-specific antibodies were seen in patients with AP and advanced destructive periodontitis, but not in LJP (Ebersole *et al.*, 1986). Furthermore, it has been suggested that *B. gingivalis* is also associated with gingivitis (Loesche & Syed, 1978; Page, 1986).

B. gingivalis fimbriae-specific antibodies were seen in PD subjects (AP, RPP and gingivitis) and to a lesser extent normal subjects aged 35–41 years but not in the 18–24-year-old group (Figs 1–3). This finding indicates that fimbriae-specific antibodies present in serum may originate from the elevated local synthesis by immunoglobulin-producing cells residing in the diseased gingival tissues. In this regard, antibodies to *B. gingivalis* have also been detected in crevicular fluid of subjects with AP (Ebersole *et al.*, 1986; Naito *et al.*, 1987). In addition, it has been shown that high concentrations of *B. gingivalis*-specific IgG and IgA antibodies were found in gingival homogenates of AP patients (Smith *et al.*, 1985). Our recent work provides further support for the local production of antigen-specific antibodies in diseased tissue (manuscript submitted). Thus, fimbriae-specific antibody secreting cells were only detectable in mononuclear cells isolated from inflamed gingiva of the patients with AP, but not in those from healthy gingiva of the same individual.

Analysis of the IgG subclass titres in *B. gingivalis* fimbriae-specific IgG antibodies revealed that antigen-specific responses were predominantly IgG3 followed by IgG1, IgG2 and IgG4 subclass in sera obtained from patients with AP, RPP and gingivitis (Fig. 2). This sequential appearance of fimbriae-specific IgG subclass antibodies suggests that the levels of the fimbriae-specific antibodies parallel the sequence $5'$–μ–$\gamma 3$–$\gamma 1$–$\alpha 1$–$\gamma 2$–$\gamma 4$–ε–$\alpha 2$–$3'$ of the immunoglobulin heavy chain genes on chromosome 4 (Franagan & Rabbits, 1982). However, our previous study showed that the enumeration of *B. gingivalis* fimbriae-specific antibody-secreting cells in mononuclear cells isolated from gingiva of AP patients gave a different pattern of IgG subclass distribution. The number of fimbriae-specific IgG subclass antibody secreting cells were elevated in the advanced stage of AP, and IgG4 and IgG1 spot-forming cells were dominant, followed by IgG3 and IgG2 (Ogawa *et al.*, 1989a). The reason for this descrepancy in IgG subclass distribution in serum antibodies and antibody-producing cells at the local disease site is at present unknown.

The increase of fimbriae-specific IgG3 and IgG1 in these patients indicates that tissue destruction which occurred in the periodontal disease lesion could be due to the aberrant production of these two antigen-specific subclass antibodies. In this regard, IgG3 and IgG1 have been shown to effectively activate the classical pathway for lysis of target cells (Burton *et al.*, 1986). Furthermore, these two IgG subclasses possess a strong binding affinity to FcγRI and FcγRII on monocytes, macrophages, NK cells and neutrophils (Unkeless *et al.*, 1988). Thus, activation of the C cascade and effector cell functions by IgG3 and IgG1 antibodies may contribute to the inflammatory response seen in local diseased sites. It was also interesting that titres of fimbriae-specific IgG4 antibody was high in sera of AP, RPP and gingivitis, but not in normal subjects (Fig. 2). This would indicate that these patients are chronically exposed to *B. gingivalis* which resulted in the induction of fimbriae-specific IgG4 antibody, since it has been suggested that prolonged exposure to protein antigens induces antigen-specific IgG4 responses (Aalberse, van der Gagg & van Leeuwen, 1983; Merrett, Burr & Merrett, 1983; Shakib, Brown & Stanworth, 1984).

Different levels and patterns of fimbriae-specific IgA1 and IgA2 antibodies were seen among PD patients tested in this study (Fig. 3). Serum samples from patients with AP and RPP had increased levels of IgA1 over IgA2 subclass responses. In general, protein antigens have been shown to induce largely IgA1 responses (Mestecky & Russell, 1986). On the other hand, although titres of antigen-specific IgA antibodies were lower in sera of patients with gingivitis, an equal distribution of IgA1 and IgA2 was noted. A similar pattern of IgA subclass response was seen in LJP and normal subjects. It is well known that IgA1 antibody is cleaved by IgA1 proteases produced by some oral bacteria (Mestecky & Killian, 1985). Thus, elevated IgA2 antibodies may represent host compensation following loss of IgA1 antibodies.

Although levels of LPS specific IgG responses in sera of AP were lower when compared with fimbriae-specific responses, the predominant LPS-specific IgG subclass antibodies were IgG2 followed by IgG4, IgG3 and IgG1 (Figs 4 and 5). This was not surprising, since it has been shown that polysaccharide antigens induce antibodies mainly of the IgG2 subclass (Hammarström & Smith, 1986). In contrast to fimbriae-specific IgA subclass distribution (e.g. IgA1 > IgA2) in sera of AP (Fig. 3a), LPS-specific IgA responses were largely seen in the IgA2 subclass (IgA1 < IgA2) (Fig. 6). IgA antibodies to LPS and to lipoteichoic acid are higher in the IgA2 subclass when compared with IgA1 in secretions (Brown & Mestecky, 1985; Mestecky & Russell, 1986). Taken together, our results provide evidence that protein and lipid carbohydrate antigens of *B. gingivalis* induce two distinct patterns of IgG and IGA subclass responses in sera of PD subjects. In this regard, fimbriae-specific responses were characterized by IgG3 > > IgG1 > IgG2 > IgG4 and IgA1 > IgA2 responses, while IgG and IgA subclass responses induced by LPS were IgG2 > > IgG4 > IgG3 > IgG1 and IgA1 < IgA2. It is intriguing that the human immunoglobulin heavy chain sequence (5′-μ-γ3-γ1-α1-γ2-γ4-ε-α2-3′), and results of the present study could suggest that at least two possible switching pathways are induced to protein *versus* carbohydrate antigens. Fimbriae antigen may induce one subset of B cells to switch subclasses which are clustered in the 5′ region of the immunoglobulin heavy chain gene including IgG3, IgG1 and IgA1. However, another switching pathway could be represented by immunoglobulin genes clustered in 3′ region (γ2-γ4-ε-α2) which seem to be predominantly involved in LPS-specific responses.

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